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(71) Applicant: XOMA CORPORATION [US/US]; 2910 Seventh Street, Berkeley, CA 94710 (US).

(72) Inventors: SCANNON, Patrick, J. ; Route 2, Box 2141, Davis, CA 95616 (US). HARMON, Richard, C. ; 2356 Tice Valley Boulevard, Walnut Creek, CA 94595 (US).

(74) Agent: WEBER, Ellen, L.; Townsend and Townsend, One Market Plaza, 2000 Steuart Tower, San Francisco, CA 94105 (US).

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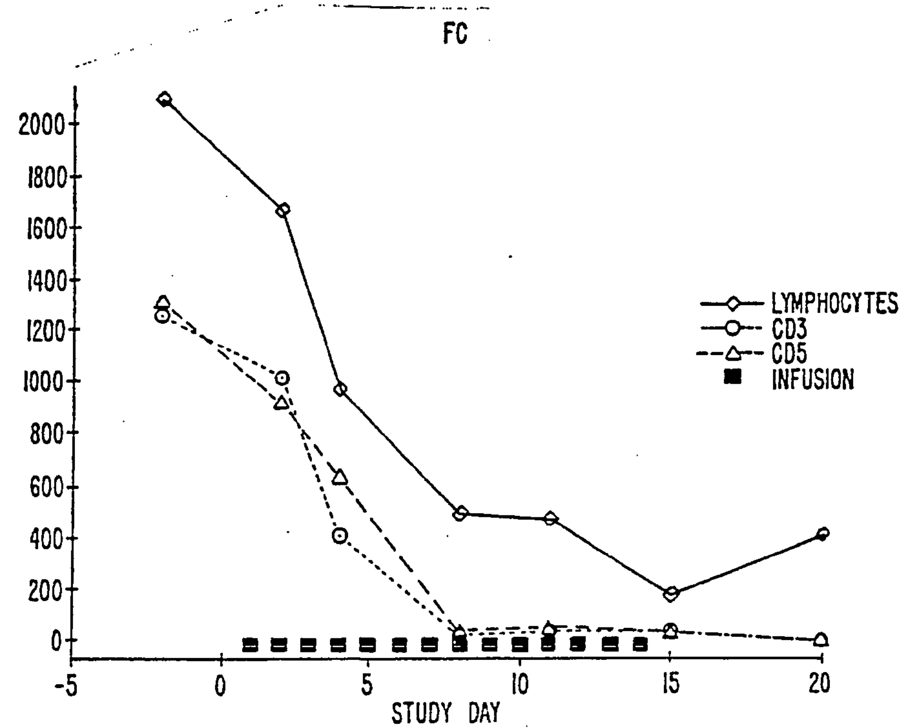

(57) Abstract

A method and therapeutic composition for the treatment of autoimmune disease by the administration of an anti-T cell immunotoxin comprising a cytotoxic agent conjugated to an anti-T cell immunoglobulin.

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THERAPEUTIC USE OF ANTI-T CELL IMMUNOTOXIN FOR AUTOIMMUNE DISEASES

BACKGROUND OF THE INVENTION

This invention relates to the treatment of autoimmune disease. In particular, this invention relates to the use of anti-T cell immunotoxins comprising a cytotoxic agent conjugated to an anti-T cell immunoglobulin for treating autoimmune disease.

The development of immunologic responsiveness of self is called autoimmunity and reflects the impairment of self-tolerance. Autoimmune diseases are pathological conditions characterized by host production of autoreactive T lymphocytes and antibodies reactive with host tissues (autoantibodies). Autoantibodies are found in some normal persons without evidence of autoimmune disease. Autoimmune diseases can occur as either organ specific or multisystem immunopathologies. Examples of autoimmune diseases include: systemic lupus erythematosus, scleroderma diseases (including lichen sclerosus, morphea and lichen planus), rheumatoid arthritis, chronic thyroiditis, pemphigus vulgaris, diabetes mellitus type 1, progressive systemic sclerosis, aplastic anemia, myasthenia gravis, myositis, Sjogrens disease, Crohn's disease, ulcerative colitis, and primary biliary cirrhosis. There is also evidence for an autoimmune association with multiple sclerosis, uveitis and Meniere's disease as well as others. A general description of various autoimmune diseases may be found in The Autoimmune Diseases, Eds., N.R. Rose & I.R. Mackey, Academic Press (1985).

The development of autoimmunity usually involves the breakdown or circumvention of self-tolerance. Normal human B cells are capable of reacting with several self-antigens (e.g. thyroglobulin) but are suppressed from producing autoantibodies by one or more mechanisms of

tolerance. Tolerance involving only T cells, induced by persistent low levels of circulating self-antigens, may be circumvented by substances such as endotoxin. Such substances would stimulate the B cells directly to produce autoantibodies, thus obviating the need for helper T cells. A decrease in suppressor T cell activity could also lead to production of autoantibodies.

Autoimmunity may be a disorder of abnormal immunologic regulation resulting in excessive B cell activity and diminished T cell activity. A decrease in suppressor T cell activity or an increase in helper T cell activity would result in uncontrolled excessive production of autoantibodies. This concept of autoimmunity is supported by studies in animal models and human autoimmune disorders in which the loss of suppressor T cell function and excessive B cell antibody production can be demonstrated.

Genetic factors may also play a role in the pathogenesis of autoimmunity. New Zealand black mice manifest autoimmune hemolytic anemia. NZB/NZW F_1 hybrids develop a disease analogous to systemic lupus erythematosus (SLE).

Several systems for differentiating between various subsets of T cells based upon cell surface antigens have been developed. The most extensive system is the so called Clusters of Differentiation or CD system. The CD system is the standardized nomenclature accepted for molecular markers of leukocyte cell differentiation molecules by the International Workshop on Human Leukocyte Differentiation Antigens. See, Leukocyte Typing III White Cell Differentiation Antigens, edited by A.J. McMichael, Oxford University Press (1987), which is incorporated by reference herein.

The CD5 cluster antigen, for example, is one of the pan T antigens present on 85-100% of the human mature T lymphocytes. It is a 67 kilodalton (KD) molecule. This antigen is not present on hematopoietic progenitor cells nor

on any other normal adult or fetal human tissue; and extensive studies by flow cytometry, immunoperoxidase staining, and red cell lysis have not demonstrated binding to such tissues. Further information regarding the CD5 marker may be found in McMichael and Gotch, "T cell antigens: new and previously defined clusters," Leucocyte Typing III, supra. The CD5 molecule has also been described in the literature as reactive with antibodies given such nomenclature. (See for example, Nancy A. Kernan et al, "Specific Inhibition of In Vitro Lymphocyte Transformation by an Anti-Pan T Cell (gp67) Ricin A Chain Immunotoxin", The Journal of Immunology 33:137-146 (1984) which is incorporated by reference herein).

CD5 positive B cells are greatly expanded in NZB mice which develop autoimmune hemolytic anemia. These same mice contribute genes to (NZB x NZW) F_1 mice, which develop a lupus-like disease.

It should also be noted that some of the immunoglobulins reactive with some of the pan T cell antigens cross react with a small number of other cells such as B cells. CD5, for instance, is also present on a small population of B lymphocytes. Several published studies indicate that B cells having a CD5 antigen produce a wide variety of the antibodies responsible for pathogenesis in at least some autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus. See, for example, P. Youinou et al., "CD5 Positive B cells in patients with Rheumatoid arthritis," Ann Rheum Dis 46: 17-22 (1987); R.R. Hardy, et al., "Rheumatoid Factor Secretion from Human Leu-1^+ B Cells, Science 236:81-83 (1987); and Casali et al., "Human Lymphocytes Making Rheumatoid Factor and Antibody to ssDNA Belong to Leu-1^+ B-Cell Subset," Science 236: 77-81 (1987), all of which are incorporated by reference herein.

According to the studies by R.R. Hardy et al., supra, B cells with the CD5 antigen typically, with some variation, constitute 20-30% of the total B cells in normal healthy humans. Individuals with rheumatoid arthritis,

Sjogrens syndrome and progressive systemic sclerosis reportedly have higher levels of B cells with the CD5 antigen than healthy individuals. Further, B cells with this antigen apparently secrete large amounts of autoantibodies.

T cell antigens other than CD5 are also present on subpopulations of B lymphocytes. For example, Small et al., J. Immuno. (1987) 138:2864-68, describe the expression of the CD1c portion of the CD1 molecule on a subpopulation of B lymphocytes. The CD1c antigen comprises a 33 kD backbone and is expressed on 14 to 46% of normal B cells derived from tonsil, peripheral blood, and spleen cells.

Monoclonal antibodies (MoAbs) directed against the CD5 antigen have been developed for use in treatment of non-autoimmune diseases such as graft versus host disease (GVHD). See for example, U.S. Patent Application Serial No. 938,855, which is incorporated herein by reference. In treatments for GVHD, such MoAbs have been conjugated by a disulfide bond to a cytotoxic ricin A chain (RTA), a ribosomal inactivating protein. See for example, U.S. Patent Application Serial No. 822,898, which is incorporated by reference herein. The MoAb targets to mature T lymphocytes and a subpopulation of B cells and causes their destruction by allowing internalization of the RTA-MoAb immunotoxin.

Chronic GVHD patients frequently present conditions and symptoms similar to certain autoimmune diseases. Chief among these are scleroderma and primary biliary sclerosis. Human patients having chronic graft versus host disease (cGVHD) provide an excellent model for the study of a number of autoimmune diseases.

Monoclonal antibodies have been used to treat autoimmune disease. For example, Wofsy, D. and Seaman, W.E., J. Exptl. Med., 161, 378 (1985) treated murine models for systemic lupus erythematosus (SLE) with weekly injections of a monoclonal antibody (MoAb) to L3T4 (CD4). (See also Wofsy, D. and Seaman, W.E., J. Immunol., 138, 3247

[1987].) Wofsy, D., J. Immunol., 136 (12), 4554 (1986) treated murine lupus in BXSB mice using rat monoclonal antibodies (MoAb) to either Thy-1.2 (on all T cells) or L3T4. MoAb Thy-1.2 retarded disease but did not prolong life. Repeated injections of MoAb to Thy-1.2 was precluded due to development of an immune response. MoAb to L3T4 was effective in treating the disease and could be readministered since there was no immune response elicited. U.S. Patent 4,695,459 also describes the treatment of experimental allergic encephalomyelitus with anti-L3T4 MoAbs and EP Application No. 85307875.6 describes the use of MoAbs to the IL-2 receptor (a 50 kd antigen) to reduce T lymphocytes in mice with heart allografts and skin grafts.

Ranges et al, Cell. Immuno., 106:163-173 (1987) reported the use of anti-L3T4 monoclonal antibodies as a preventative and, in some cases, therapeutic treatment for several murine models of autoimmune disease. Proliferative response was inhibited only when the antibodies were administered prior to immunization. Treatments with anti-L3T4 antibodies were ineffective in modulating ongoing humoral responses, particularly when they were administered one week or more after immunization.

Benjamin, R.J., et al., J. Exp. Med., 163, 1539 (1986) studied the complication of an antiglobulin response in MoAb therapy. Such a complication has two aspects. The first is to curtail the prospect of long-term therapy. The second is the potential harmful hypersensitivity reactions. These authors concluded that the antiglobulin response is a major complication of MoAb therapy. (This complication has in fact been confirmed in the therapeutic use of murine monoclonal antibodies as immunosuppressive or anti-tumor agents, e.g., OKT3. [Chatenoud, L. et al., J. Immunol., 137, 830 (1986)].)

Doney et al., MoAb Exp. Hematol. 13:520-524 (1985) administered MoAb compositions comprising two MoAbs reactive with peripheral T cell antigens to eleven with aplastic anemia. None of the patients responded to the therapy.

Gould, B., et al., International Symposium on Immunotoxins, June 9, 1988, describe the continuous infusion of an immunotoxin for the treatment of metastatic breast cancer. The monoclonal antibody-directed to a breast tumor antigen was conjugated to ricin A chain. Significant toxicities were observed.

U.S. Patent 4,550,086 describes monoclonal antibodies capable of binding to a surface recognition structure of a predetermined mature human T cell clone. They propose that the recognition structure renders the clone capable of acting as a causative agent in a particular autoimmune disease. MoAbs to that recognition structure neutralized the clone in vitro.

Kim, Y.W. and Metzgar, R.S., International Symposium on Immunotoxins, June 9, 1988, administered an immunotoxin to tumor bearing mice. The immunotoxin included an anti-pancreatic tumor antibody conjugated to pokeweed antiviral protein.

An object of the present invention is to provide a therapeutic composition for the treatment of autoimmune disease. Examples of such autoimmune diseases include: systemic lupus erythematosus, scleroderma diseases (including lichen sclerosus, morphea and lichen planus), rheumatoid arthritis, chronic thyroiditis, pemphigus vulgaris, diabetes mellitus type 1, progressive systemic sclerosis, apalastic anemia, myasthenia gravis, myositis, Sjogrens disease, Crohn's disease, ulcerative colitis, and primary biliary cirrhosis. Another object of this invention is to provide a method of treatment of such autoimmune disorders.

SUMMARY OF THE INVENTION

The present invention is based on the novel and unexpected observation that an anti-T cell immunotoxin can be administered safely and effectively in the treatment of autoimmune disease. In one aspect, the invention is directed to administration of a pharmaceutical composition

comprising an anti-T cell immunotoxin to patients having an autoimmune disease. It has been observed for the first time that the therapeutic benefit, to a patient having an autoimmune disease, of administration of an anti-T cell immunotoxin is sustained well beyond the time of administration. In another aspect the invention is directed to a method of treatment of autoimmune disease. More specifically the anti-T cell immunotoxin comprises a cytotoxic molecule such as a lectin A chain conjugated to an immunoglobulin reactive with a T cell.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the depletion of the lymphocyte population with the CD5 marker in two patients with cGVHD resulting from a 14 day course of injections of XMMLY-H65-RTA.

Fig. 2 shows the depletion of the lymphocyte population with the CD3 marker in three patients with cGVHD resulting from a 14 day course of injections of XMMLY-H65-RTA.

Fig. 3 summarizes the results of lymphocyte cell depletion in a patient who was administered the subject immunotoxins of Figs. 1 and 2.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, "anti-T Cell Immunotoxin" refers to an immunotoxin capable of therapeutically improving the medical condition of a patient with autoimmune disease. The anti-T cell immunotoxin comprises a cytotoxic molecule and a T-lymphocyte reactive component. The cytotoxic molecule is typically fatal to a cell. The T cell reactive component binds to a T-lymphocyte and is capable of delivering the cytotoxic molecule to that T-lymphocyte. While the immunoglobulin portion of the immunotoxin is referred to as anti-T cell, it is the case that the immunoglobulin is not exclusively reactive with T cells.

The T-lymphocyte reactive component of the immunotoxin is an immunoglobulin which can be obtained from a number of sources and which is reactive with a T cell specific antigen. The term "immunoglobulin(s)" includes polyclonal antibodies, monoclonal antibodies, reactive fragments thereof, such as Fv, Fab, $F(ab)_2$, synthetic immunoglobulins and recombinant immunoglobulins including chimeric immunoglobulins or their derivatives. Preferably, the immunoglobulins are MoAbs of the IgM or IgG isotype of murine, human or other mammalian origin which are reactive with most T cells or with both T cells and subsets of other lymphoid cells, such as B cells or NK cells. More preferably the MoAbs are reactive with one or more of several T cell markers including CD2, CD3, CD5 and CD7. Most preferably the MoAb is reactive with the CD5 antigen found on both T and B cells. It would be understood that MoAbs of other animal species could be prepared using analogous non-human mammalian markers.

For this invention, an immunoglobulin is "reactive" with an antigen when the immunoglobulin interacts with the antigen. This interaction is analogous to a chemical reaction in which two reactants come together to form a product. In the case of the immunoglobulin antigen interaction, the product of the interaction is an antigen-immunoglobulin complex. The preferred antigen is generally a unique surface protein, including various types of receptors, produced by or displayed by T cells or a subset of T and other lymphoid cells. However, a large variety of antigens, such as other proteins, glycoproteins, lipoproteins, polysaccharides and the like which are produced by or displayed by the cells and recognized by the immunoglobulin, can be utilized in accordance with the present invention. The T cell specific antigens useful in the present invention are antigens or markers typically found on most T cells present in a human patient. Preferred markers are the CD2, CD3, CD5 and CD7 antigens. Other antigen clusters such as

CD6 could also serve as sources for T cell specific antigens. A most preferred marker is the CD5 antigen cluster.

It is also noted that some of the anti-T cell immunoglobulins, which may be utilized in the present invention, cross react with a small number of other lymphoid cells, such as B cells. Anti-CD5 immunoglobulins, for instance, react with T cells and with a small sub-population of B cells. Further, the immunotoxin preparation may be composed of two or more immunoglobulins, each reactive with a different marker or the same marker or different cell populations to ensure a broad spectrum of T cell neutralization.

A variety of methodologies are presently known in the art for producing MoAbs. See, e.g., Goding, Monoclonal Antibodies; Principles and Practice, Academic Press, 2nd Edition (1986), which is incorporated herein by reference. Less preferred forms of immunoglobulins may be produced by methods well-known to those skilled in the art; e.g., chromatographic purification of polyclonal sera to produce substantially monospecific antibody populations. The most preferred monoclonal antibody for use in this invention is that produced by hybridoma cell line XMMLY-H65 deposited with the A.T.C.C. and given the Accession No. HB9285. The antibody portion of the preferred immunotoxin is prepared as described in the U.S. Patent Application Serial No. 974,824, page 14, lines 25-36. The antibody is preferably activated for coupling to the preferred cytotoxin, RTA, with SPDP (as described in U.S. 4,590,071, column 4, line 55 to column 5, line 5, except that the buffer contains 5% dextrose instead of azide).

A variety of cytotoxic molecules are suitable for use in immunotoxins. The cytotoxic molecules contemplated by this invention can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatinum; and cytotoxic proteins such as ribosomal inhibiting proteins including, pokeweed antiviral

protein, abrin and ricin (or their A-chains). Functionally equivalent are the ADP ribosylatine toxins such as diphtheria toxin and Pseudomonas exotoxin A, etc. or their recombinant derivatives. See generally, "Chimeric Toxins", Olsnes and Pihl, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), both of which are incorporated herein by reference.

Toxic lectins are of particular interest in this invention. The cytotoxic action of toxic lectins, and especially that of ricin and abrin, has been well studied. It is known that toxic lectins consist of two polypeptide chains, A and B, linked by means of disulfide bridge(s). Cytotoxicity is associated with the A chain and its inhibition of protein synthesis in nucleated cells. The B chain is essentially a delivery vehicle for the A chain. The B chain recognizes polysaccharide units at the surface of cells and creates a high affinity interaction with such units. Once the B chain binds with polysaccharide units at the cell surface, the A chain is incorporated into the cell, blocking ribosomal protein synthesis and ultimately leading to cell death. The use of ricin A chain is preferred in this invention.

Toxic lectins of the type of structure and function similar to ricin include abrin, modeccin and mistletoe toxin. One other category of ribosomal inactivating protein (RIP) is the toxin with only one subunit having functional characteristics analogous to ricin A chain. This type of RIP lacks cytotoxicity to the intact cell because of the inherent absence of a binding fragment analogous to ricin B chain. Examples of RIP's of this latter type include gelonin and pokeweed antiviral protein.

According to the invention, any toxic lectin which may be split into A and B polypeptide chains, specifically abrin, modeccin and mistletoe toxin may be used in the same way ricin is used in the preferred embodiment. In addition, any RIP or equivalent ADP ribosylating toxin specifically

gelonin, pokeweed antiviral protein, may be used in the same way as ricin A chain. Such materials are equivalent to the toxic lectin A chain for purposes of this invention.

It is preferable in this invention to achieve cell specific cytotoxicity by employing toxic A chains that are substantially free of lectin B chain impurities. Preferred methods for obtaining substantially pure lectin A chains are described in U.S. Patent Application Serial No. 829,544 and in U.S. Patent Application Serial No. 053,189, which are both incorporated herein by reference. Toxic lectins may be prepared using recombinant means.

The most preferred ricin toxin A chain for use in this invention is one wherein substantially pure RTA-30 is used. The term "RTA-30" refers to a species of ricin toxin A chain having a molecular weight of approximately 30 kD, such as described in detail by Fulton et al. J. Biol. Chem., 281:5314-5319 (1986) and Vidal et al. Int. J. Cancer, 36:705-711 (1985). For the purposes of this invention, RTA preparations containing concentrations of about 75% or more of RTA-30 are considered substantially pure. Preparation of substantially pure RTA-30 for use in conjunction with a MoAb is described in U.S. Patent Application Serial Number 074,824 which is incorporated herein by reference.

Methods for conjugating the immunoglobulin to the lectin A chain are also described in U.S. Patent Application Serial No. 053,189 at page 7, lines 18-37 and page 8, lines 1-10. A particularly comprehensive disclosure of conjugation methodologies may be found in European Patent Application Publication No. 169111 which is incorporated by reference herein. A preferred method of conjugation is the formation of a covalent, disulfide bond between the immunoglobulin and the toxic lectin A chain.

The two components are complexed or chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic molecule is a protein and the second component is an intact immunoglobulin, such as a monoclonal antibody, the linkage

may be by way of carbodiimide, glutaraldehyde, heterobifunctional cross-linkers, e.g., N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) and derivatives, 2-iminothiolane and derivatives, homobifunctional cross-linkers, e.g., bis-maleimide, cross-linking of proteins without exogenous cross-linkers by means of groups reactive to the individual protein such as carbohydrate, disulfide, carboxyl or amino groups via oxidation or reduction of the native protein, or treatment with an enzyme or the like. Production of various immunotoxins is well-known within the art, and can be found, for example, in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet", Thorpe et al. Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), which is incorporated herein by reference. The RTA solution and the antibody solution are preferably combined as described in U.S. Serial No. 974,824, page 15, lines 1-9 and the immunotoxin elutes as fractions (as described in U.S. 4,590,071, column 5, lines 15-24). TWEEN 80 may be added up to 0.1% in the final solution.

Commercially prepared XMMLY-H65-RTA (XOMAZYME-H65-RTA) produced by Xoma Corporation, Berkeley, California may alternatively be used as the immunotoxin embodying a preferred immunotoxin preparation of this invention. This preparation is a conjugate of RTA and a MoAb reactive with the CD5 antigen.

The immunotoxin may be administered to a patient either singly or in a cocktail containing two or more immunotoxins, other therapeutic agents, compositions, or the like, including, but not limited to, immunosuppressive agents, potentiators and side-effect relieving agents. Of particular interest are immunosuppressive agents useful in suppressing allergic reactions of a host. Immunosuppressive agents of interest include prednisone, DECADRON (Merck, Sharp & Dohme, West Point, PA), cyclophosphamide, cyclosporine, 6-mercaptopurine, methotrexate, azathioprine and i.v. gamma globulin or their combination. Potentiators of interest include monensin, ammonium chloride and

chloroquin. All of these agents are administered in generally accepted efficacious dose ranges such as those disclosed in the Physician Desk Reference, 41st Ed. (1987), Publisher Edward R. Barnhart, New Jersey. A commonly assigned U.S. Application Serial No. 07/151,741 discloses administration of an immunotoxin as an immunosuppressive agent and is incorporated by reference herein.

For use in the invention herein, anti-T cell immunotoxins may be formulated into either an injectable or topical preparation. Parenteral formulations are known and are suitable for use in the invention, preferably for i.m. or i.v. administration. The formulations containing therapeutically effective amounts of anti-T cell immunotoxins are either sterile liquid solutions, liquid suspensions or lyophilized versions and optionally contain stabilizers or excipients. Lyophilized compositions are reconstituted with suitable diluents, e.g., water for injection, saline, 0.3% glycine and the like, at a level of about from .01 mg/kg of host body weight to 10 mg/kg where the biological activity is less than or equal to 20 ng/ml when measured in a reticulocyte lysate assay. Typically, the pharmaceutical compositions containing anti-T cell immunotoxins will be administered in a therapeutically effective dose in a range of from about .01 mg/kg to about 5 mg/kg of the treated animal. A preferred therapeutically effective dose of the pharmaceutical composition containing anti-T cell immunotoxin will be in a range of from about 0.01 mg/kg to about 0.5 mg/kg body weight of the treated animal administered over several days to two weeks by daily intravenous infusion, each given over a one hour period, in a sequential patent dose-escalation regimen.

Anti-T cell immunotoxin is formulated into topical preparations for local therapy by including a therapeutically effective concentration of anti-T cell immunotoxin in a dermatological vehicle. The amount of anti-T cell immunotoxin to be administered, and the anti-T cell immunotoxin concentration in the topical formulations, will

depend upon the vehicle selected, the clinical condition of the patient, the systemic toxicity and the stability of the anti-T cell immunotoxin in the formulation. Thus, the physician will necessarily employ the appropriate preparation containing the appropriate concentration of anti-T cell immunotoxin in the formulation, as well as the amount of formulation administered depending upon clinical experience with the patient in question or with similar patients. The concentration of anti-T cell immunotoxin for topical formulations is in the range of greater than from about 0.1 mg/ml to about 25 mg/ml. Typically, the concentration of anti-T cell immunotoxin for topical formulations is in the range of greater than from about 1 mg/ml to about 20 mg/ml. Solid dispersions of anti-T cell immunotoxin as well as solubilized preparations can be used. Thus, the precise concentration to be used in the vehicle will be subject to modest experimental manipulation in order to optimize the therapeutic response. Greater than about 10 mg anti-T cell immunotoxin/100 grams of vehicle may be useful with 1% w/w hydrogel vehicles in the treatment of skin inflammation. Suitable vehicles, in addition to gels, are oil-in-water or water-in-oil emulsions using mineral oils, petrolatum and the like.

Anti-T cell immunotoxin optionally is administered topically by the use of a transdermal therapeutic system (Barry, 1983, Dermatological Formulations, p. 181 and literature cited therein). While such topical delivery systems have been designed largely for transdermal administration of low molecular weight drugs, by definition they are capable of percutaneous delivery. They may be readily adapted to administration of anti-T cell immunotoxin or derivatives thereof and associated therapeutic proteins by appropriate selection of the rate-controlling microporous membrane.

Topical preparations of anti-T cell immunotoxin either for systemic or local delivery may be employed and may contain excipients as described above for parenteral

administration and other excipients used in a topical preparation such as cosolvents, surfactants, oils, humectants, emollients, preservatives, stabilizers and antioxidants. Any pharmacologically acceptable buffer may be used, e.g., tris or phosphate buffers. The topical formulations may also optionally include one or more agents variously termed enhancers, surfactants, accelerants, adsorption promoters or penetration enhancers, such as an agent for enhancing percutaneous penetration of the anti-T cell immunotoxin or other agents. Such agents should desirably possess some or all of the following features as would be known to the ordinarily skilled artisan: be pharmacologically inert, non-promotive of body fluid or electrolyte loss, compatible with anti-T cell immunotoxin (non-inactivating), and capable of formulation into creams, gels or other topical delivery systems as desired.

Anti-T cell immunotoxin may also be administered by aerosol to achieve localized delivery to the lungs. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing or derivatives thereof. Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of anti-T cell immunotoxin together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers will vary depending upon the requirements for the particular anti-T cell immunotoxin, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. The formulations also can include mucolytic agents as well as bronchodilating agents. The formulations will be sterile. Aerosols generally will be prepared from isotonic solutions. The particles optionally include normal lung surfactants.

Aerosols may be formed of the particles in aqueous or nonaqueous (e.g., fluorocarbon propellant) suspension. Such particles include, for example, intramolecular

aggregates of anti-T cell immunotoxin or derivatives thereof or liposomal or microcapsular-entrapped anti-T cell immunotoxin or derivatives thereof. The aerosols should be free of lung irritants, i.e., substances which cause acute bronchoconstriction, coughing, pulmonary edema or tissue destruction. However, nonirritating absorption enhancing agents are suitable for use herein. Sonic nebulizers preferably are used in preparing aerosols. Sonic nebulizers minimize exposing the anti-T cell immunotoxin or derivatives thereof to shear, which can result in degradation of anti-T cell immunotoxin.

Anti-T cell immunotoxin may be administered systemically, rather than topically, by injection i.m., subcutaneously, intrathecally or intraperitoneally or into vascular spaces, particularly into the joints, e.g., intraarticular injection at a dosage of greater than about 1 μg/cc joint fluid/day. The dose will be dependent upon the properties of the anti-T cell immunotoxin employed, e.g., its activity and biological half-life, the concentration of anti-T cell immunotoxin in the formulation, the site and rate of dosage, the clinical tolerance of the patient involved, the autoimmune disease afflicting the patient and the like as is well within the skill of the physician.

The anti-T cell immunotoxin of the present invention may be administered in solution. The pH of the solution should be in the range of pH 5 to 9.5, preferably pH 6.5 to 7.5. The anti-T cell immunotoxin or derivatives thereof should be in a solution having a suitable pharmaceutically acceptable buffer such as phosphate, tris (hydroxymethyl) aminomethane-HCl or citrate and the like. Buffer concentrations should be in the range of 1 to 100 mM. The solution of anti-T cell immunotoxin may also contain a salt, such as sodium chloride or potassium chloride in a concentration of 50 to 150 mM. An effective amount of a stabilizing agent such as an albumin, a globulin, a gelatin, a protamine or a salt of protamine may also be included and may be added to a solution containing anti-T cell

immunotoxin or to the composition from which the solution is prepared.

Systemic administration of anti-T cell immunotoxin is made daily, generally by intramuscular injection, although intravascular infusion is acceptable. Administration may also be intranasal or by other nonparenteral routes. Anti-T cell immunotoxin may also be administered via microspheres, liposomes or other microparticulate delivery systems placed in certain tissues including blood. Topical preparations are applied daily directly to the skin or mucosa and then preferably occluded, i.e., protected by overlaying a bandage, polyolefin film or other barrier impermeable to the topical preparation.

The method is illustrated by way of the following examples, which are not to be construed as limiting the invention.

EXAMPLE 1

Preparation of Anti-T Cell Immunotoxin

A preferred production process for RTA-based immunotoxins is described in U.S. Patent No. 4,590,071, which is incorporated herein by reference.

a. Ricin Extraction from Whole Castor Beans.

Whole castor beans are mechanically ground, and ricin extracted from the meal with a solution of 0.9% saline. This solution was filtered from the bean pellet and lipid layer using a Celite Filter Aid and Aerosil Adsorbent (Manville Denver, CO; Degussa, Frankfurt, W. Germany). The filtrate was concentrated and then diafiltered against Tris lactose, pH 7.8 (50 mM lactose, 10 mM Tris pH 7.8, 50 mM NaCl), and passed through a QAE ZETA prep cartridge (AMF-Cuno, Meriden, Connecticut, LKB Instruments, Pleasant Hill, CA). The resultant material was diafiltered against a Tris saline solution (10 mM Tris, 0.9% NaCl, pH 7.8).

b. Ricin Toxin A Chain Separation.

The diafiltrate was applied to a Sepharose 4B column (Pharmacia Fine Chemicals, Piscataway, NJ) and the

nonbinding flow-through containing ricin was loaded onto an acid-treated Sepharose column in order to separate the ricin toxin A chain from the whole ricin (as described in U.S. 4,590,071, column 3, lines 26-52). The eluant thus obtained was diafiltered against Tris buffer (10 mM Tris, 10 mM NaCl), and the resulting filtrate was passed through a QAE Sepharose Fast Flow column (Pharmacia Fine Chemicals) equilibrated to the same buffer. The RTA obtained above was adjusted in NaCl concentration to 0.9 wt.%, and purified to remove toxin B chain impurities by applying to a Sepharose column previously coupled to goat anti-ricin toxin B chain antibodies.

c. Immunotoxin Preparations.

The murine monoclonal antibody XMMLY-H65 is reactive with the human CD5 antigen. The cell line XMMLY-H65 was deposited with the A.T.C.C. and designated Accession No. HB9286. Immunotoxins utilizing that monoclonal antibody were prepared as follows:

An XMMLY-H65 tissue culture harvest was concentrated and the pH adjusted to 8.5. The solution was applied to an immobilized Staph. Protein A Column and eluted with 0.1 M Citrate, pH 4.5. The eluate was diafiltered against 10 mM Hepes Buffer, 0.25 M NaCl, pH 7.3, and then applied to a QAE Sepharose Fast Flow column. The antibody passed through the column, and was diafiltered against PBS, pH 7.0, 5% dextrose. The antibody was activated for coupling to the RTA with SPDP (as described in U.S. 4,590,071, column 4, line 55, column 5, line 5, except that the buffer contained 5% dextrose instead of azide).

A concentrated RTA-30 solution and the antibody solution were placed together in a formulation buffer consisting of 10 mM PO_4, pH 7.0, 0.15 M NaCl, and 5% dextrose. This solution was applied to a Sephacryl S-200 HR column (Pharmacia Fine Chemicals, location?), which had been pre-equilibrated with PBS containing 5% dextrose, and the immunotoxin eluted as fractions (as described in U.S.

4,590,071, column 5, lines 15-24). In some batches, TWEEN 80 was added up to 0.1% in the final solution.

EXAMPLE 2

Treatment of Autoimmune-like Diseases Associated With Chronic Graft Versus Host Disease

The Anti-T cell immunotoxin XOMAZYME H65 (XMMLY-H65-RTA) has been employed in the treatment of cGVHD patients as part of FDA approved clinical trials of that drug for the treatment of cGVHD patients. The following summarizes the results of the therapy of several GVHD patients with scleroderma and primary biliary sclerosis-like conditions. Scoring is based on a normal value of 0 progressing to advanced disease at 4+.

a. Patient FC.

Patient FC was a 35 year old male with aplastic anemia. He received a bone marrow transplant (BMT) from an HLA identical sister. He developed acute GVHD within one month after transplant and progressed to chronic GVHD. At time of immunotoxin therapy, he was on a high dose of methyl prednisolone and a moderate dose of Cyclosporin A. His chronic GVHD had been unresponsive, with 100% involvement of skin with open pustules, oral stomatitis, and a large ulcerated hard palate. On immunotoxin, the skin showed definite softening and oral stomatitis and the hard palate ulcers resolved. He was able to have Cyclosporine A and methyl prednisolone tapered to low levels. Chronic GVHD then flared up again in 6 months.

Table 1. Results of immunotoxin therapy for patient FC.

Day:	0	7	15	28-40
SKIN	3+	3+	2+	2+
GUT	2+	1+	1+	1+
LIVER	1+	1+	1+	1+

XMMLY-H65-RTA dose = 0.05 mg/kg/day for 14 days.

b. Patient GH.

Patient GH was a 47 year old female with chronic myelogenous leukemia (CML). She received BMT from an HLA identical brother. She developed acute GVHD 3 months post-transplant and subsequently developed chronic GVHD. She was being treated with moderate methyl prednisolone and moderate Cyclosporine A. Her disease was refractory to treatment, with severe scaling, puritis, and increased pigmentation of the skin. Within 7 days of immunotoxin treatment, scaling had decreased 50%, and skin showed softening and decreased pigmentation. Improvement continued through day 40. The patient was maintained on moderate methyl prednisolone (no Cyclosporine A) for 3 months with stabilization of the disease. Within 6 months she was being tapered off all immunosuppressants.

Table 2. Results of immunotoxin therapy for patient GH.

Day:	0	7	15	28-40
SKIN	3+	2+	1+	1+
GUT	0	0	0	0
LIVER	0	3+	1+	1+

XMMLY-H65-RTA dose = 0.1 mg/kg/day for 14 days.

c. Lymphocyte depletion.

Lymphocyte population was decreased in patients with cGVHD by administration of immunotoxin therapy.

Patients FC and BM were each administered XMMLY-H65-RTA intravenously for a 14-day course in a sequential patient dose escalation regimen ranging from 0.05 mg/kg/day through 0.33 mg/kg/day for 14 days. Each dose was given over a one-hour period. From the date of infusion and over the course of 20 days in the case of patient FC, and the course of 28 days in the case of patient BM, lymphocytes were isolated from whole blood and analyzed for the presence of CD5 markers using the immunofluorescence method to analyze the cells by flow cytometry. Fig. 1 shows the

marked depletion of lymphocytes with the CD5 marker in each patient resulting from the immunotoxin treatment.

Patients FC, MS and BM with cGVHD, in a manner similar to that described in paragraph 3(a) above, were administered XMMLY-H65-RTA. The lymphocyte cell population with the CD3 marker was determined as above over the course of 20, 24 or 28 days (depending on the patient) from the date of initial infusion. Fig. 2 shows the marked depletion of lymphocytes with the CD3 marker in each patient resulting from the immunotoxin treatment. Fig. 3 shows the results in patient FC of the depletion of the lymphocytes with the CD3 marker, those with the CD5 marker and of all lymphocytes. The depletion of CD5 and CD3 marker cells approaches zero, while the total depletion of lymphocytes, though significantly decreased drops to about 200 cells/ml at day 15.

EXAMPLE 3

Anti-T Cell Immunotoxin Treatment of Rheumatoid Arthritis

Patients having rheumatoid arthritis were selected for treatment using an anti-T cell immunotoxin of this invention. Patients must have had definite rheumatoid arthritis (RA) for at least four years. Active disease was assessed using criteria known to the ordinarily skilled physician. These include, for example: multiple (10 or more) grade 6 swollen joints (on a 0-10 scale); pain and tenderness in multiple joints; morning stiffness greater than 30 minutes; erosive changes on X-ray characteristic of RA; poor response to progressively aggressive therapy, having failed oral anti-inflammatory drugs, injectable gold and low dose oral steroid therapy. Patients were taken off all disease modifying agents for approximately one month prior to treatment with the anti-T cell immunotoxin of this invention.

XomaZyme®-H65 prepared as described above was administered at doses of 0.05, 0.1, 0.2 and 0.33 mg/kg/day for a period of 5-9 days. Four patients were treated at

each dose. Patients were monitored using several indicia including joint selling and tenderness scores. Patients responded at each of the dosage levels. One patient who received XomaZyme®-H65 at .05 mg/kg/day has for 6 months shown significant therapeutic benefit. Similar sustained effects with a single treatment regimen over 5-9 days at the 0.1 mg/kg/day (two patients) and at 0.33 mg/kg/day (one patient) was observed. It has been observed for the first time that the therapeutic benefit, to a patient having an autoimmune disease, of administration of an anti-T cell immunotoxin is sustained well beyond the time of administration.

EXAMPLE 4

Anti-T Cell Immunotoxin Treatment of Severe Acute Aplastic Anemia

Aplastic anemia is a life-threatening disorder characterized by pancytopenia and bone marrow hypocellularity. There is a large body of data supporting the view that severe acute apalastic anemia is an autoimmune disease.

Patients are selected for treatment who are diagnosed as having moderate or severe aplastic anemia. That diagnosis is established as follows: a bone marrow biopsy showing hypoplasia (decrease of 10% cellularity); and granulocyte (<500/mm^3), platelets (<20,000/mm^3) or reticulocyte (<90,000/mm^3) counts as indicated.

XomaZyme®-H65 is prepared as described above. Patients are administered 10 infusions of XomaZyme®-H65 over one hour on ten successive days. The anti-T cell immunotoxin is administered at doses of 0.2, 0.33 and 0.5 mg/kg/day. Patients are monitored for response to therapy by improvement in granulocytes and platelet counts or transfusion requirements at 3 and 6 months post therapy.

EXAMPLE 5

Anti-T Cell Immunotoxin Treatment of Type I Diabetes

There are two major types of diabetes. Type I has classically been associated with the requirement of exogenous insulin. Type I typically occurs before the age of 40 and is associated with an absence of insulin secretion. The pancreas of patients with long-term type I insulin dependent diabetes are devoid of pancreatic islet cells. There is a large body of evidence that the etiology of type I insulin dependent diabetes (IDDM) is autoimmune.

Patients are diagnosed as having IDDM based on the criteria established by the American Diabetes Association.

XomaZyme®-H65 is prepared as described above. Patients are administered 5 infusions of XomaZyme®-H65 over one hour on five successive days. The anti-T cell immunotoxin is administered at doses of 0.1 and 0.2 mg/kg/day. Patients are monitored for response to therapy by measuring insulin levels required to maintain normal or acceptable fasting glucose levels.

Using diagnostic criteria predictive of the onset of Type 1 diabetes, patients are selected for prophylactic treatment. Patients are administered anti-T cell immunotoxin at doses about 0.1 and about 0.2 mg/kg/day.

Although the present invention has been described in some detail by way of example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for treating autoimmune disease comprising administration of a therapeutically effective dose of an anti-T cell immunotoxin comprising a cytotoxic molecule conjugated to an anti-T cell immunoglobulin to an animal having an autoimmune disease.

2. The method of claim 1, wherein the cytotoxic molecule is a ribosomal inhibiting protein (RIP) or ADP ribosylating toxin.

3. The method of claim 2, wherein the RIP is a toxic lectin A chain.

4. The method of claim 3, wherein the toxic lectin A chain is a ricin A chain (RTA).

5. The method of claim 4, wherein the RTA is RTA-30.

6. The method of claim 1, wherein the anti-T cell immunoglobulin is reactive with at least one T cell marker.

7. The method of claim 1, wherein the autoimmune disease is rheumatoid arthritis.

8. The method of claim 1, wherein the autoimmune disease is diabetes mellitus type 1.

9. The method of claim 1, wherein the autoimmune disease is aplastic anemia.

10. The method of claim 1, wherein the autoimmune disease is inflammatory bowel disease.

11. The method of claim 10 wherein the inflammatory bowel disease is Crohn's disease.

12. The method of claim 10 wherein the inflammatory bowel disease is ulcerative colitis.

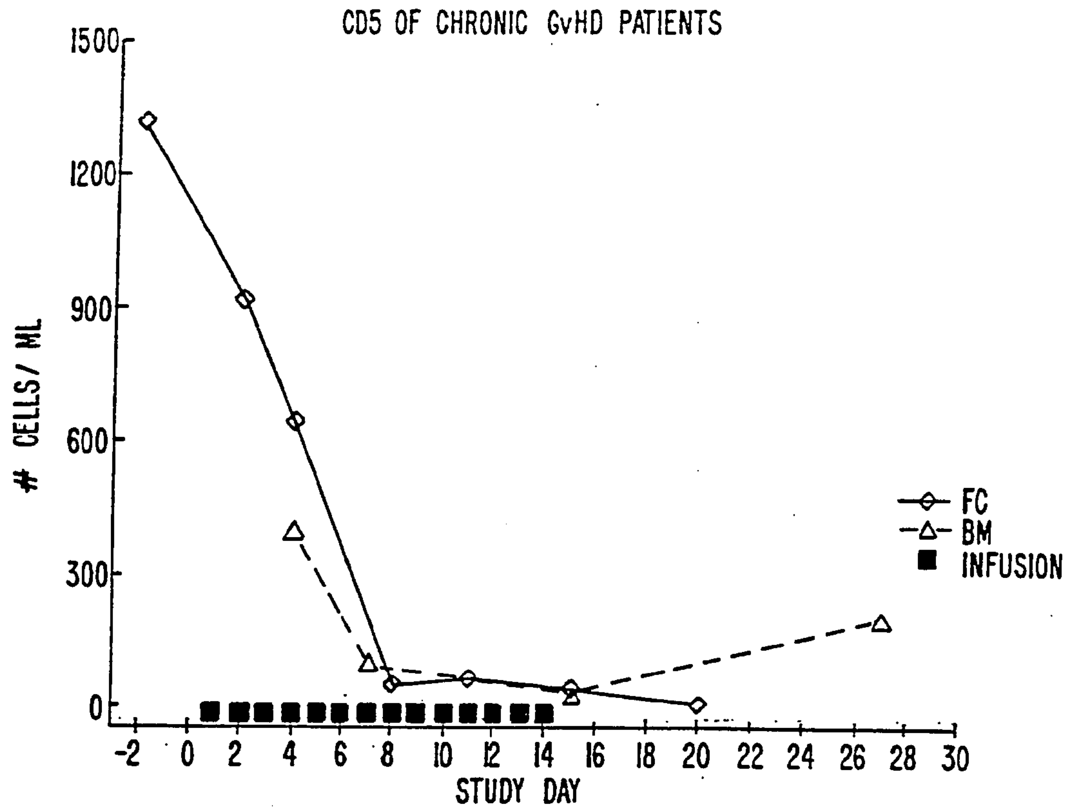
13. The method of claim 1, wherein the anti-T cell immunoglobulin is a monoclonal anti-T cell antibody.

14. The method of claim 13, wherein the monoclonal antibody is that produced by hybridoma XMMLY-H65 having A.T.C.C. Accession No. HB9286.

15. The method of claim 13, wherein the monoclonal antibody is murine.

16. The method of claim 1, wherein the anti-T cell immunotoxin is administered in a therapeutically effective dose of from about 0.01 mg/kg to about 5 mg/kg of host body weight.

17. The method of claim 1, wherein the anti-T cell immunotoxin is administered in a therapeutically effective dose of from about .05 mg/kg to about 0.33 mg/kg of host body weight.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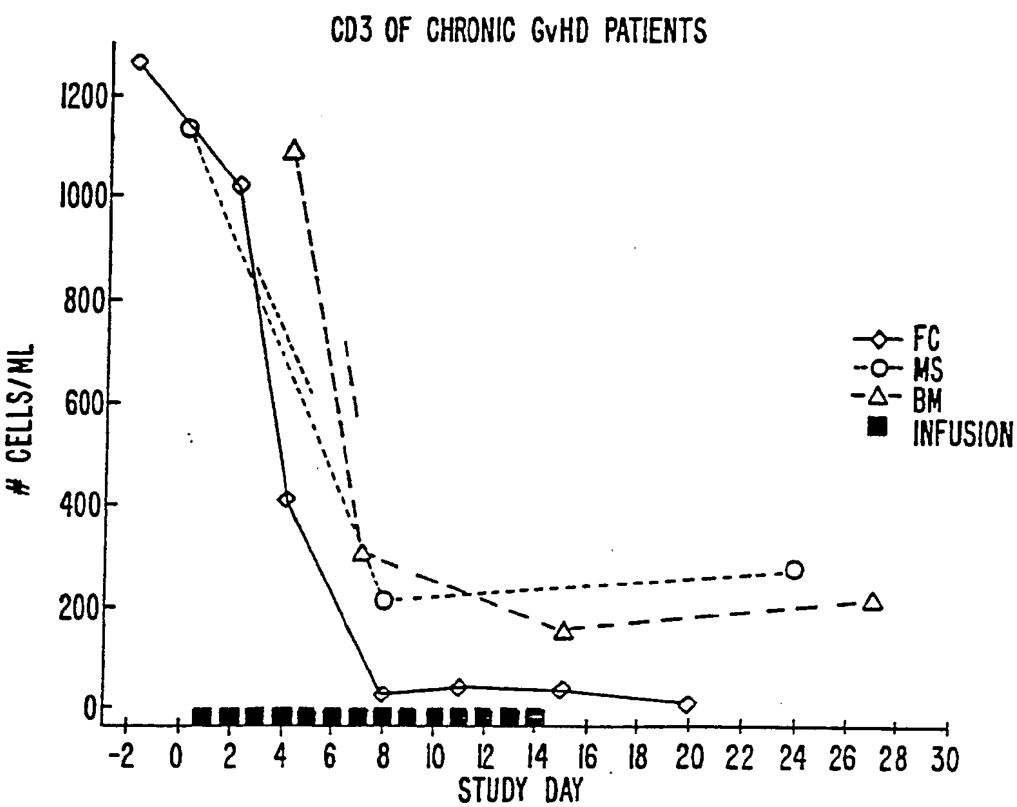
STUDY DAY

FIG._1.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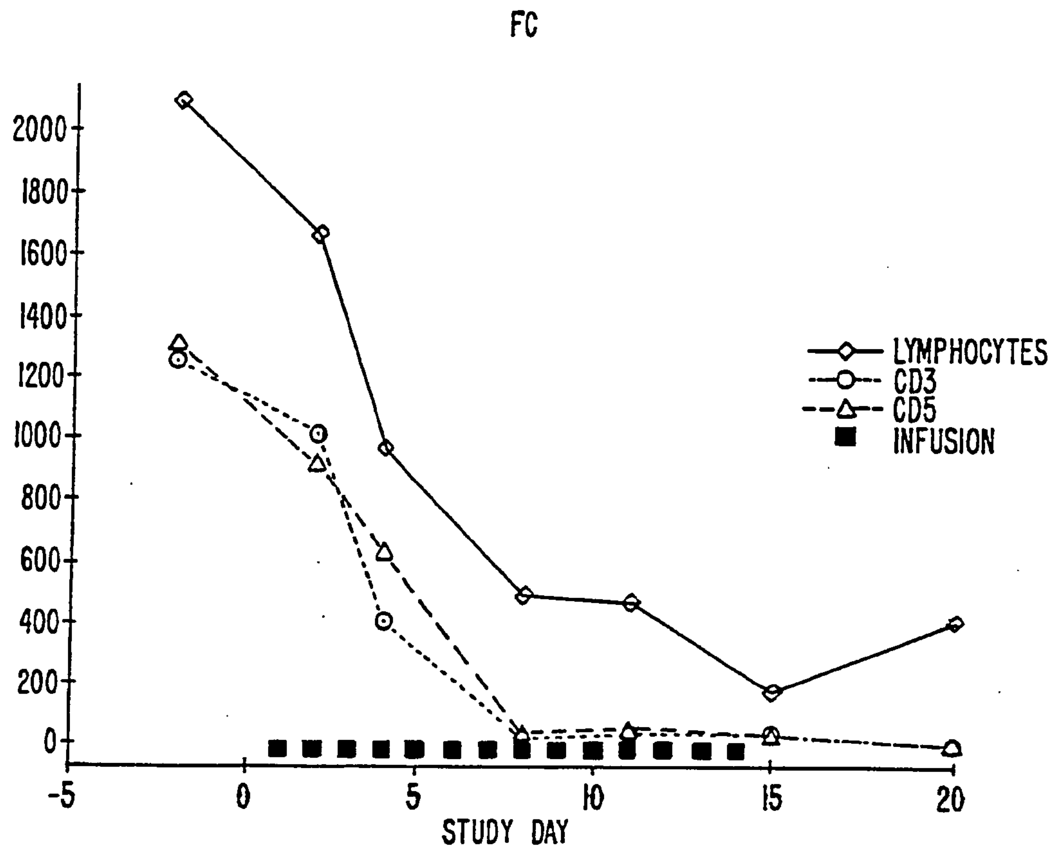
STUDY DAY

FIG._2.



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LYMPHOCYTES
CD3
CD5
INFUSION

FIG._3.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00440

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl. A61K 37/00, 37/24, 39/00, 39/395, 45/02

US Cl. 424/85.91,85.8,85.1,85.2,85.4; 514/2,8,12,21,814,885,886,825,866

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US	424/85.91,85.8,85.1,85.2,85.4, 514/2,8,12,21,814,885,886,925,926,927,928,825,866

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X / Y	British Journal of Heamatology, Vol. 67, Issued 1987, "Elemination of T-cells from Human Peripheral Blood and Bone Marrow Using a Cocktail of Three Anti-T-Cell Immunotoxin," (Katz), pages 407-11, See page 407.	1-6,13 / 7-12,14-17
X	Blut, Vol. 50, Issued 1985, "Ex Vivo Elemination of of Neoplastic T-Cells from Human Marrow Using an Anti-M_r-41,00 Protein Immunotoxin:Potentiation by ASTA Z7557," (Uckun), pages 19-23, See page 19	1-6,13 / 7-12,14-17
Y	Cancer Research, Vol. 47, Issued January 1987, "Combined Immunochemotherapy of Human Solid Tumors in Nude Mice," (Weil-Hillman), pages 579-85, See page 579.	1-17
Y	Cancer Research, Vol. 45, Issued November 1985, "Kinetics of Protein Synthesis Inactivation in Human T-Lymphocytes by Selective Monoclonal Antibody-Ricin Conjugates," (Leonard), pages 5263-5269, See page 5263.	1-17
P,Y	US, A, 4,731,244, (Talle), 14 March 1988, See claims.	1-17

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 April 1989	07 JUN 1989
International Searching Authority	**Signature of Authorized Officer**
IPEA/ISA	GARNETTE D. DRAPER

Form PCT/ISA/210 (second sheet) (Rev.11-87)